Ultraviolet B Irradiation Induces Changes in the Distribution and Release of Arachidonic Acid, Dihomo-γ-linolenic Acid, and Eicosapentaenoic Acid in Human Keratinocytes in Culture

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There is increasing evidence that derivatives of 20-carbon polyunsaturated fatty acids, the eicosanoids, play an important role in the inflammatory responses of the human skin. To better understand the metabolic fate of fatty acids in the skin, the effect of ultraviolet B (UVB) irradiation (280–320 nm) on the distribution and release of ^{14}C-labeled arachidonic acid, dihomo-γ-linolenic acid, and eicosapentaenoic acid in human keratinocytes in culture was investigated. Ultraviolet B irradiation induced the release of all three ^{14}C-labeled fatty acids from the phospholipids, especially from phosphatidylethanolamine, and this was accompanied by increased labeling of the nonphosphorus lipids. This finding suggests that UVB induces a significant liberation of eicosanoid precursor fatty acids from cellular phospholipids, but the liberated fatty acids are largely reincorporated into the nonphosphorus lipids. In conclusion, the present study suggests that not only arachidonic acid but also dihomo-γ-linolenic acid, and eicosapentaenoic acid might be involved in the UVB irradiation-induced inflammatory reactions of human skin. *J Invest Dermatol 88:611–614, 1987*

Excessive exposure of the human skin to ultraviolet B (UVB) irradiation is followed by cutaneous erythema, which is apparently a result of the action of a variety of inflammatory mediators including the oxygenated derivatives of arachidonic acid [1]. As a result of UV irradiation, the concentrations of both arachidonic acid and its metabolites are elevated in the skin [2–4]. In addition to arachidonic acid, both dihomo-γ-linolenic acid and eicosapentaenoic acid may also affect the inflammatory reactions of the skin [5,6]. So far, the studies on the effect of UVB irradiation on the membrane fatty acid metabolism have been almost entirely restricted to the metabolism of arachidonic acid. Therefore, in the present study the effect of UVB irradiation on the distribution and release of all three eicosanoid precursor fatty acids was investigated.

MATERIALS AND METHODS

Materials and procedures employed were essentially the same as described earlier [7] unless indicated.

Cell Culture, Labeling, UVB Irradiation, and Cell Viability

In this study a keratinocyte cell line NCTC 2544 from Flow Laboratories was used. This cell line originates from a clone of skin cells derived from a white male [8]. Several lines of evidence indicate that the NCTC 2544 cells are nonmalignant: the cells form a monolayer in culture, exhibit growth inhibition, and by light microscopy the nuclei have been found not to appear malignant [9]. Moreover, the cells have been found not to grow in soft agar as most malignant cells have been reported to do [9]. Positive staining of the NCTC 2544 cells with mouse monoclonal antihuman cytokeratins (Dakopatts, CK-1, dilution 1:100), in combination with immunofluoresence staining (goat antimouse IgG, Vector Laboratories), has been demonstrated in our laboratory. The cells were cultured (40×10^6 cells per each flask) in a medium of 40 ml containing 90% RPMI 1640 (KC Biological), and 10% fetal calf serum (Flow Laboratories). In the beginning of the labeling, 10 ml of the culture medium was replaced with an equal volume of fresh medium containing 700,000 cpm (about 6.6 nmol) of one of the ^{14}C-labeled fatty acids, respectively. Following the labeling period of 24 h, the medium containing unincorporated radioactivity was removed, and the cells were washed with a small volume of fresh Hanks' buffered salt solution (HBSS). For the UVB irradiation or the corresponding sham treatment (covered with opaque material), the cells were detached from the culture flasks and transferred in a 10 ml volume of HBSS (without phenol red) into Petri dishes. The cells were irradiated at room temperature for 10 s, 1 min, or 12 min, respectively, at a distance of 20 cm from a bank of fluorescent tubes (Airam LUB) emitting in the 280–400 nm range (maximum at 315 nm) with a UVB irradiance of 1.157 mW/cm^2 (corresponding to 0.145 mW/cm^2 of erythemally effective (EE) UVB, weighted at 296.7 nm). The intensity of the irradiation in the UVC range (wavelengths shorter than 280 nm) was less than 1% of the intensity of UVB irradiation. Following the irradiation, 30 ml of RPMI was added to the culture dishes and incubations were stopped 24 h after the irradiation.

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Abbreviations:

HBSS: Hanks' buffered salt solution
PLA_2: phospholipase A_2
TLC: thin-layer chromatography
UVB: ultraviolet B (280–320 nm)

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For the subsequent lipid analysis, the cells were detached by gentle mechanical rubbing without trypsin, and separated from the culture medium by centrifugation. Cell viability was determined by trypan blue exclusion assay 24 h after the irradiation. The percentage of viable cells was 90 ± 2%, 84 ± 2%, 72 ± 3%, 64 ± 3%, in control experiments, following 1.5 mJ/cm^2, 9 mJ/cm^2, and 104 mJ/cm^2, respectively.

Analysis of the Cellular Lipids The distribution of radioactivity in different lipid fractions was analyzed as follows [7]. Keratinocytes were homogenized in 10 ml of chloroform:methanol (2:1, v:v) containing 0.01% of α-tocopherol to prevent oxidation, and the homogenate was filtered through glass wool. Following mixing 0.2 vol of 0.12 M KCl with the filtrate, the organic phase was removed, and the sample was evaporated to dryness under nitrogen. Subsequently, 6 ml of acetone saturated with $MgCl_2$ was added to the residue, and the tubes were stored overnight at −20°C. The tubes were centrifuged, the supernatant containing the neutral lipids was removed, and the neutral lipids were thereby separated from the precipitated phospholipids. After acetone was evaporated into dryness, both the neutral lipid and phospholipid fractions were redissolved in chloroform.

Neutral lipid and phospholipid fractions were analyzed separately by 2 different thin-layer chromatography (TLC) systems [7]. The lipid fractions were identified by unlabeled standards that were visualized by exposure to iodine vapor. The distribution of radioactivity on the TLC plates was checked by autoradiography of the TLC plates. The amount of radioactivity was determined by liquid scintillation counting, and the values were corrected for the recovery of the analysis. To measure the amounts of radioactivity released into the culture medium, 1-ml samples of the culture medium were taken for liquid scintillation counting.

Enzymatic Treatment of Phospholipids To determine positional specificity of the esterified ^{14}C-labeled fatty acids, individual phospholipid classes were treated with bee venom phospholipase A_2(PLA_2, EC 3.1.1.4, Sigma, St. Louis, Missouri). The phospholipids were separately scraped off from the TLC plates and extracted into chloroform:methanol:acetic acid:water (50:39:1:10, v:v:v:v). The fractions treated with PLA_2 were dried under nitrogen, redissolved in 0.2 ml of diethyl ether, and incubated for 48 h at 30°C with 10 μl of bee venom stock solution (0.5 mg lyophilized bee venom in 0.5 ml 0.1 M Tris-HCl, pH 7.4, 5 mM $CaCl_2$). Following PLA_2 hydrolysis, the samples were analyzed by TLC as described for the neutral lipids. In this system unhydrolyzed phospholipids remain at the origin while free fatty acids released by the PLA_2 migrate with a greater R_f value.

Statistical Analysis of the Data Following inverse sine (arcsine) transformation the present data were subjected to one-way analysis of variance. After observing the overall significance in one-way analysis of variance, the comparisons of treatments with the control group were performed using the Bonferronis test.

Table I. The Effect of UVB Irradiation on the Distribution of ^{14}C-Labeled Fatty Acids Within Cellular Lipids

		UVB mJ/cm^2 (Erythemally Effective)			
		0	1.5	9	104
PE	20:3	34.2 ± 1.4	34.1 ± 1.2	26.4 ± 1.6*	24.9 ± 2.8*
	20:4	41.9 ± 2.1	45.3 ± 1.9	35.9 ± 1.5	32.9 ± 2.8*
	20:5	44.7 ± 3.6	44.6 ± 1.1	34.1 ± 1.9*	32.1 ± 0.9*
PC	20:3	18.6 ± 2.8	15.9 ± 0.5	12.9 ± 0.5	12.0 ± 1.2*
	20:4	10.8 ± 1.6	12.8 ± 0.4	12.6 ± 2.2	10.7 ± 1.1
	20:5	12.5 ± 2.0	12.0 ± 1.7	9.4 ± 0.9	9.9 ± 0.4
PI+PS	20:3	18.3 ± 1.9	16.7 ± 1.2	19.5 ± 0.4	19.1 ± 2.1
	20:4	17.0 ± 0.9	12.7 ± 0.9	12.7 ± 0.9	18.1 ± 2.3
	20:5	7.3 ± 0.7	6.8 ± 0.6	9.2 ± 0.5	6.7 ± 0.6
SM	20:3	0.3 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
	20:4	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
	20:5	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.1
PA	20:3	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
	20:4	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
	20:5	0.3 ± 0.1	0.4 ± 0.1	0.5 ± 0.2	0.4 ± 0.1
TG	20:3	3.9 ± 0.5	3.8 ± 0.4	7.1 ± 0.2**	11.4 ± 0.6***
	20:4	2.7 ± 0.2	2.1 ± 0.1	4.8 ± 0.3***	7.1 ± 0.4***
	20:5	4.6 ± 0.4	4.1 ± 0.4	8.4 ± 0.4***	11.5 ± 0.2***
DG	20:3	1.2 ± 0.1	1.0 ± 0.1	1.2 ± 0.2	1.4 ± 0.1*
	20:4	0.8 ± 0.1	1.1 ± 0.1	0.8 ± 0.1	1.2 ± 0.1
	20:5	0.7 ± 0.1	0.7 ± 0.1	1.1 ± 0.2**	1.3 ± 0.1***
CE	20:3	1.0 ± 0.1	0.9 ± 0.1	1.4 ± 0.1*	2.1 ± 0.1***
	20:4	0.8 ± 0.1	0.7 ± 0.1	1.2 ± 0.1***	1.9 ± 0.1***
	20:5	1.9 ± 0.1	1.9 ± 0.1	2.3 ± 0.3	2.9 ± 0.2**
FFA	20:3	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.1***
	20:4	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.1***
	20:5	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.1**	0.6 ± 0.1***

The cells were labeled for 24 h with one of the ^{14}C-labeled fatty acids before UVB irradiation. The distribution of radioactivity was analyzed 24 h after UVB irradiation. The results are expressed as percentage of the total amount of the initially incorporated radioactivity (mean ± SEM, n = 3–4). The statistical significances were calculated using two-way analysis of variance and the treatments were compared to the controls using the Bonferronis test: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Abbreviations:
PE: phosphatidylethanolamine
PC: phosphatidylcholine
PI+PS: phosphatidylinositol + phosphatidylserine
SM: sphingomyelin
PA: phosphatidic acid
TG: triacylglycerols
DG: diacylglycerols
CE: cholesteryl esters
FFA: free fatty acids

RESULTS

Following a labeling period of 24 h 68%, 72%, and 67% of [^{14}C]arachidonic acid (20:4n6), [^{14}C]dihomo-γ-linolenic acid (20:3n6), or [^{14}C]eicosapentaenoic acid (20:5n3), respectively, was incorporated into the human keratinocytes in culture. Most of the radioactivity was recovered in different phospholipids, and the largest amounts of radiolabel were found in phosphatidylethanolamine (Table I). The percentage amount of the incorporated [^{14}C]eicosapentaenoic acid in the nonphosphorus lipids was slightly greater than that of dihomo-γ-linolenic acid or arachidonic acid (Fig 1). All three eicosanoid precursor fatty acids were obviously predominantly bound into the 2-positions of the phospholipids (Table II).

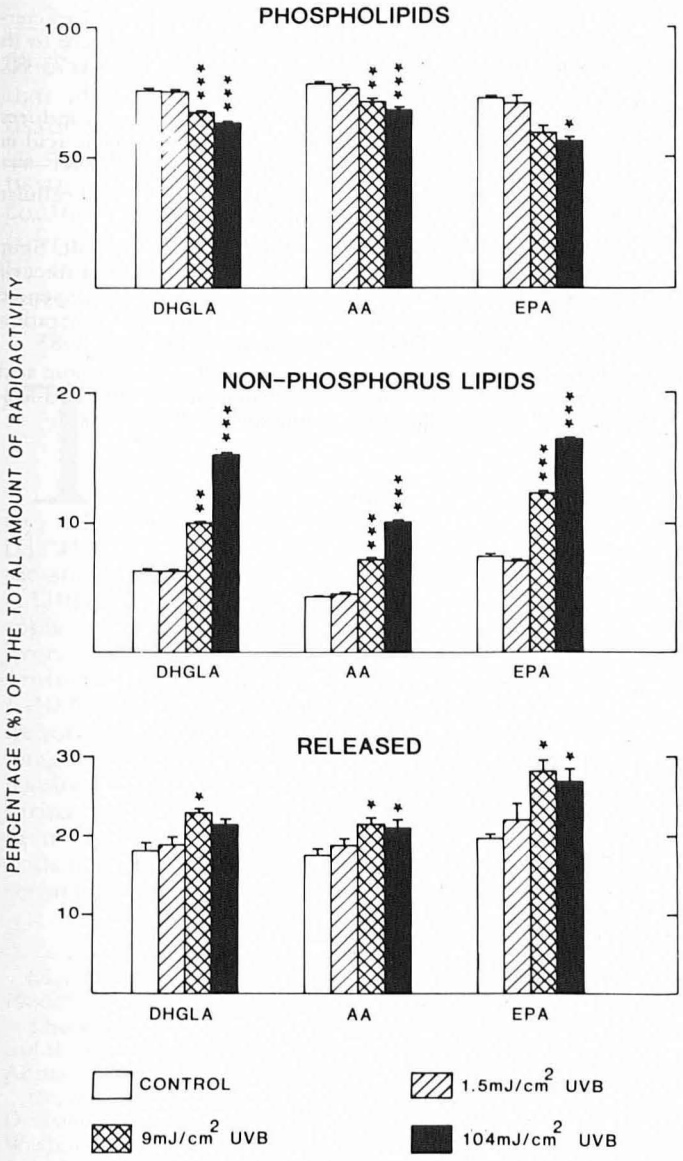

Figure 1. The effect of UVB irradiation on the amounts of [^{14}C]-dihomo-γ-linolenic acid (*DHGLA*), [^{14}C]-arachidonic acid (*AA*), and [^{14}C]-eicosapentaenoic acid (*EPA*) released into the culture medium, incorporated into the nonphosphorus lipids, and incorporated into the phospholipids. The keratinocytes were first labeled for 24 h with one of the ^{14}C-labeled fatty acids and then UVB irradiated. The distribution of radioactivity was analyzed 24 h thereafter and the results are expressed as percentage (mean ± SEM, n = 3–4) of the total amount of radioactivity incorporated into the cells following the labeling. After observing the overall significance in one-way analysis of variance, the comparisons of treatments to the control were performed using the Bonferronis test: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. The doses of irradiation are expressed as mJ/cm^2 of erythemally effective (EE) UVB, weighted at 296.7 nm.

Table II. Release of the ^{14}C-Labeled Fatty Acids From Phosphatidylcholine (PC), Phosphatidylethanolamine (PE), and Phosphatidylinositol Plus Phosphatidylserine (PI + PS) by Bee Venom Phospholipase A_2 (PLA_2)

		20:3n6	20:4n6	20:5n3
PC	Not released	3.7	4.2	4.4
	Released by PLA_2	96.3	95.8	95.6
PI + PS	Not released	3.2	3.6	3.6
	Released by PLA_2	96.8	96.4	96.4
PE	Not released	3.5	5.2	4.3
	Released by PLA_2	96.5	94.8	95.7

The results are expressed as percentage of the total amount of radioactivity and are the means of 3 analyses giving closely related results.

The distributions of dihomo-γ-linolenic acid and arachidonic acid in the cellular lipids were rather similar (Table I). Compared with arachidonic acid, however, slightly smaller amounts of dihomo-γ-linolenic acid were found in phosphatidylethanolamine, whereas the amounts found in phosphatidylcholine were slightly larger. Eicosapentaenoic acid was incorporated into the phosphatidylinositol + phosphatidylserine fraction less efficiently than either arachidonic acid or dihomo-γ-linolenic acid.

Following a 24-h culture after the labeling, part of the incorporated ^{14}C-labeled fatty acids was spontaneously released into the culture medium. Ultraviolet B irradiation stimulated the release of these eicosanoid precursor fatty acids into the culture medium (Fig 1). The distribution of these fatty acids within the cellular lipids was also changed following UVB irradiation: the ^{14}C-labeling of phosphatidylethanolamine showed decrements whereas the labeling of the nonphosphorus lipids (triacylglycerols and cholesteryl esters) showed increments. Unlike arachidonic acid and eicosapentaenoic acid, dihomo-γ-linolenic acid was released in addition to phosphatidylethanolamine also from phosphatidylcholine.

DISCUSSION

Radiolabeled eicosanoid precursor fatty acids were effectively incorporated into the human keratinocytes in culture and the bulk of the radioactivity was found in different phospholipids. Ultraviolet B irradiation induced release of all three eicosanoid precursor fatty acids and the major phospholipid source of the released ^{14}C-labeled fatty acids was phosphatidylethanolamine. Following UVB irradiation, eicosapentaenoic acid was released more readily than dihomo-γ-linolenic acid or arachidonic acid. Interestingly, eicosapentaenoic acid was also incorporated least effectively into the phospholipids. Following UVB irradiation the precursor fatty acids were mainly liberated from phospholipids, into the 2-positions to which they were predominantly bound, suggesting that phospholipase activities, probably PLA_2, are involved in the UVB irradiation-induced release of fatty acids. Nonspecific events related to membrane perturbation and cell damage may, however, also interfere with the release of fatty acids [10]. Ultraviolet B irradiation induced the release of radioactivity from phospholipids, which was accompanied by an increase in the labeling of the nonphosphorus lipids. Thus, part of the liberated fatty acids was apparently reincorporated into the nonphosphorus lipids.

Exposure of the human skin to UVB irradiation is followed by an inflammatory reaction in which metabolites of arachidonic acid are suggested to play an important role [1,4]. As dihomo-γ-linolenic acid, and eicosapentaenoic acid may together account for an even higher proportion of fatty acids than arachidonic acid, it is possible that also dihomo-γ-linolenic acid and eicosapentaenoic acid might have an important role in both physiologic and pathophysiologic reactions of the skin. Furthermore, dihomo-γ-

linolenic acid and eicosapentaenoic acid may serve as substrates for the same metabolic routes as arachidonic acid, which means that they may also interfere with the formation of metabolites of arachidonic acid [11–13].

In summary, UVB irradiation induces changes in distribution and release of arachidonic acid, dihomo-γ-linolenic acid, and eicosapentaenoic acid in human keratinocytes in culture. As the availability of free fatty acid obviously is the rate-limiting step in the formation of eicosanoids, it appears that in addition to arachidonic acid either dihomo-γ-linolenic acid or eicosapentaenoic acid might also affect the UVB-induced inflammatory reactions of the human skin.

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